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The surprising anatomical diversity in the roots of African Restionaceae

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Abstract

The root anatomy of 291 of the 350 species of the African Restionaceae, one of the ecologically dominant and taxonomically diverse elements of the Cape flora, is reported. There is substantial variation in the cortex (either collapses in older roots or persists as an aerenchyma), endodermis (in relative size, shape of the endodermal cells, and degree of wall thickening), pericycle (from 1— to 10 cells, varying from unthickened to massively thickened), and the metaxylem vessels (5 to more than 100, organized in a ring or scattered). Almost all root anatomical characters are phylogenetically constrained, similar to the culm anatomical characters. Although it is possible to, based on root anatomy, recognize groups of genera with broadly similar root anatomies, there are often also exceptions, indicating some evolutionary lability. Variation not phylogenetically controlled is significantly explained by differences in ground water availability and mean annual precipitation. The descriptive data are available in an online interactive key. The variation in root anatomy may contribute interesting insight into the evolution of this unusual group of plants.

Keywords: Anatomy; Cape flora; Evolution; Phylogenetic constraint; Restionaceae; Roots

1 Introduction

Belowground structures and organs of plants are poorly investigated, except for agriculturally important species. This is surprising, as in many regions access to water and nutrients may be more limiting than access to light and CO_2, and consequently we can expect that the belowground structures should be functionally and phylogenetically at least as important as above-ground vegetative organs. The importance of belowground structures is corroborated by the plants themselves, which may allocate up to 70% of photosynthate to roots (Poorter et al., 2012; Valverde-Barrantes et al., 2017). Variation patterns in roots, in both functional and morphological-anatomical traits, are complex, varying ontogenetically from young to old roots, architecturally within one plant, and at all phylogenetic scales. Simplistically, roots may be interpreted to consist of two functional domains. The “fine roots” or the root-hair zone, directly behind the growing root tip and so the youngest part of the roots, are actively involved in water and nutrient uptake. Fine root functional traits include root length, N content, weight, root hair development and fungal associations (Lambers et al., 2006), and are part of the “plant economic spectrum” that combines root and shoot traits (Freschet et al., 2015; Kramer-Walter et al., 2016; Pohl et al., 2011). Fine root functional traits show both phylogenetic and ecological signals, root traits may even be more phylogenetically constrained than leaf traits (reviewed in Valverde-Barrantes et al., 2017). This is formulated as the “root trait phylogenetic conservatism hypothesis”. The older part of the roots is primarily a transport pipe, moving water from the root tips to the above-ground part of the plant, and nutrients and oxygen below ground to the fine roots, this transport is often through a hostile environment. Mature root functional traits include metaxylem vessel size (Lynch et al., 2014) and organization, as well as aerenchyma and the controls on radial oxygen loss (Connell et al., 1999).

It is unclear how much older root anatomical variation is phylogenetically or ecologically constrained, and consequently how useful this could be systematically. Although there is a general survey of root anatomical structure and variation across the monocots, as part of the Anatomy of Monocots project (e.g. Cutler, 1969; Metcalfe, 1960; Tomlinson, 1969), there have been relatively few detailed (e.g., almost complete generic coverage) systematic comparisons focusing on families or smaller groups. Seubert (1996a, b, 1997, 1998a, b, 1997, 1998a,b) surveyed the roots of palms, and found little variation among congeneric species, but substantial variation between genera. Similarly, Carlquist (1966) found that in Rapateaceae congeneric species and related genera are quite similar in their root anatomy, but that there are systematic differences between groups of genera. Wilder (1986) also found that generally the genera of Cyclanthaceae could be grouped based on the root anatomy, but that some of these groupings were unexpected, based on other characters. That species groups within a genus can also be determined is evident from the study on the roots of *Allium* (Fritsch, 1992). However, a survey of bambusoid root anatomy revealed substantial anatomical variation, which was not taxonomically structured (Raechal and Curtis, 1990). This is not consistent with a global grass root anatomy survey, which suggested that there was a phylogenetic pattern in the variation (Goller, 1977). Such a phylogenetic pattern was also demonstrated by a careful study by Uma and Muthukumar (2014) in the Zingiberaceae. A very common problem, mentioned by almost all researchers working on a comparative analysis of root anatomy (e.g. Carlquist, 1966; Cutler, 1969; Metcalfe, 1960) is that the sampling, at species level, is too poor. Kauff et al. (2000) introduced the idea that root anatomy could be adapted to dry conditions in Asparagales. A convincing case study, with root anatomy well sampled at species level, and interpreted in both phylogenetic and ecological context, is needed to evaluate the systematic and ecological importance of mature root anatomical variation.

The absence of root information applies particularly to the Cape flora. This is surprising, as the species-rich *fynbos* flora is found in a seasonally arid climate on nutrient-poor soils, in an environment with no shortage of light. Rain falls in winter, when it is too cold to grow, whereas the warm summers are dry (Cramer and Hoffman, 2015). In such an environment competition should be largely belowground, and affect the roots: deep rooted plants can access water in summer, shallow-rooted cannot (West et al., 2012). The suggestion that competition may be largely belowground leads to the prediction that there should be substantial variation in root anatomy in the Cape flora clades. Root traits may be adaptive to extreme soil conditions, thus convergent (see Tanentzap and Lee, 2017), or they could be phylogenetically constrained. Restionaceae is highly diverse in the Cape flora (Linder, 2003) and one of the dominant elements of the typical *fynbos* vegetation (Rebelo et al., 2006). The species are often separated along hydrological gradients (Araya et al., 2012), and this has been linked to the presence of aerenchyma in the roots (Huber and Linder, 2012). The root anatomy of the Australian Restionaceae has been surveyed (Meney et al., 1999; Pate and Delfs, 1999). Shane et al. (2011) demonstrated the role of the sand-sheaths in *Lyginia* R.Br.. The *Lyginia* root system is quite remarkable, with 2–4 m deep roots reaching the permanent water-table, whereas shallower roots are summer-dormant (Shane et al., 2009). However, very little has been published on roots of the African Restionaceae (the monophyletic subfamily Restionoideae (Briggs and Linder, 2009), which we will refer to as restios): Cutler (1969) described the root anatomy of 18 ~~African Restionaceae~~restio species (Table S1), and Huber and Linder (2012) documented the distribution of aerenchyma.

Here we describe the anatomy of the roots of the restios. We first develop the descriptive terminology, and interpret the ontogenetic development of the root anatomical tissues. This is based on a small number of species to document the development of the anatomy from the root tip to the mature roots. Then we used a large sample of roots from herbarium specimens, and some freshly collected material, to document the variation in the root anatomy across the subfamily. We then map this variation across the phylogeny and test which traits are phylogenetically conserved. We test whether the phylogenetic signal differs from that of the culm anatomy. Restio~~naceae~~ culm anatomy has long been interpreted to be phylogenetically and systematically informative (Cutler, 1969; Gilg-Benedict, 1930; Gilg, 1891; Linder, 1984), and the culm anatomy has been scored from almost all restio species (Linder, 2001), making such a comparison possible and interesting. We use these root traits to diagnose the genera of the restios. Finally, in order to test whether species with similar root anatomy are found in similar environments, we simplified the root anatomical variation to four ordination dimensions, thus generated abstract root functional ~~traits~~syndromes. We tested whether (and which) environmental variables significantly explained variation in these functional ~~traits~~syndromes after phylogenetic correction.

2 Materials and ~~M~~methods

2.1 Sampling

In order to understand and map the root anatomical diversity of the restios, we followed three approaches. First, in order to explore the ontogeny of the root tissues, and so to establish the homologies among the roots, we studied 10 species (*Cannomois grandis* H.P.Linder, *Elegia elephantina* H.P.Linder, *E. fistulosa* Kunth, *Restio leptostachyus* Kunth, *R. paniculatus* Rottb., *Rhodocoma capensis* Nees ex Steud., *Thamnochortus bachmannii* Mast., *T. cinereus* H.P.Linder, *T. spicigerus* (Thunb.) Spreng. and *Willdenowia incurvata* (Thunb.) H.P.Linder) in detail. These were cultivated in the botanical garden of the University of Zurich, facilitating the collection of undisturbed 1~~st~~st order roots. The plants were grown in normal potting soil and with a biweekly watering regime, under glass to prevent freezing, for at least two years to ensure that mature roots were present. The roots were fixed in formaldehyde:ethanol:acetic acid (4%:50%:5%) for at least 48 h~~ours~~, before being stored in 70% EtOH. Although sampling was constrained by the species available in cultivation, we were able to select pairs of closely related species, such that the species-pairs come from phylogenetically widely separated genera. Secondly, in order to control for sectioning artifacts, re-hydrated roots of 42 species from seven genera (harvested from herbarium specimens, see Table S1) were embedded in 2-hydroxyethyl methacrylate (Technovit7100; Heraeus Kulzer GmbH, Wehrheim, Germany), following the manufacturer's protocol, and sectioned at 3.5–10 µm with a Rotary Microtome HM355S. The sections were stained with Ruthenium Red and Toluidine Blue and mounted with Histomount.

Finally, to document the variation across the whole subfamily, we attempted to include all restio species. Roots were sampled from herbarium specimens in the combined herbaria of the University of Zurich (Z) and the Federal Technical University (ZT), as well as the Bolus Herbarium of the University of Cape Town (BOL). Where possible several collections per species were sampled, and in all cases the identity of the specimen was verified. We collected about 1 cm of dried root. Although we could not determine exactly where along the root the material was sampled, almost all samples were from the 1st order roots, and in the mature part (usually within 5 cm from the plant base). Because they are monocots without secondary thickening, it may not be so important to locate the exact places the root is sampled. We assume that if several phylogenetically closely related species show the same characters that this is characteristic of the species, and not a plastic response. In addition, we also field collected some species into FAA. This survey resulted in data for 298 of the 350 species, with all genera represented by at least one species (Table S1)

2.2 Sectioning methods

Herbarium material was incubated in a water-soap solution for three days, and preserved in 70% EtOH. Roots were sectioned transversally with a razor blade under a dissecting microscope, double-stained with Alcian blue (1% in H_2O) and Safranin (1% in EtOH) (2:1) (Tolivia and Tolivia, 1987). Alcian blue stains compounds with anionic groups blue and Safranin stains cellulose and lignin red. Sections were dehydrated through a sequence of increasing EtOH concentrations, washed in butanol, transferred to Histoclear, and mounted in Histomount on glass slides. Drying takes one month in the drying oven at 25 °C. The sections were visualized with a photomicroscope (Axioskop2 MOT, with

AxioCam HRc) at magnifications of 40~~to~~ 200 times. Scale bars were added to the pictures with AxioVision4.8. The slides are in the Department of Systematic and Evolutionary Botany of the University of Zurich.

2.3 Data scoring

All slides were studied under an Olympus CH2, using bright light. Data were scored and organized in Delta (Dallwitz, 1980; Dallwitz and Paine, 1986). This allowed data to be kept as unordered or ordered multistate (categorical) data, as count values (meristic), or as real continuous data. Data were exported for phylogenetic analyses using the NEXUS format (Maddison et al., 1997) that is readily imported into Mesquite. Data were also exported into the Intkey format (http://delta-intkey.com/www/programs.htm). ~~Descriptive data, at species level, available in Delta Intkey at http://www.systbot.uzh.ch/en/Bestimmungsschluessel/Restionaceae.html.~~

2.4 Phylogeny

I explored the phylogenetic structure in the root anatomical data using the maximum clade credibility tree of the restios from Bouchenak-Khelladi and Linder (2017). This phylogenetic tree is based on 10~~,~~121 aligned plastid DNA basepairs for 335 species (98% of African restios). I used the Drop.Tip function implemented in R package 'Ape' (Paradis et al., 2004) to remove species for which no root anatomical data were available, this left 291 species of the 350, representing all genera and subgenera of the African Restionaceae.

Root anatomical character variation was mapped and visualized in Mesquite 3.31 (Maddison and Maddison, 2017) using parsimony optimization. I first tested whether each character evolved in a phylogenetically conservative fashion. In order to do this, I randomly reshuffled the states of each character 1000 times. This keeps the proportion among the character states, as well as the character model. The observed number of steps needed to fit the character on the tree is then compared to the distribution of steps. This constitutes a tail probability test, which I interpreted to be one-sided (the observed number of steps should always be less then randomized number of steps). I assigned * as fewer than 100 trees, ** fewer than 50 and *** as fewer than 10 trees. This test is implemented in Mesquite 3.31.

In order to select those characters which are most consistent with the phylogeny, I also calculated the retention index (RI) (Farris, 1989), which quantifies the phylogenetic signal each character gives (calculated as the ~~(~~maximum number of changes on a tree minus the observed number of changes on the tree~~)~~, ~~and~~ divided~~ing~~ by the ~~(~~maximum number of changes on the tree minus the minimum number of changes in the dataset). I also calculated the consistency index (CI) as (minimum number of possible changes in the character, divided by the observed number of changes). The two were summarized as the rescaled consistence (RC) index (RI x CI). These calculations were implemented in Mesquite. To determine whether the phylogenetic signal in root anatomical characters is stronger than that of culm anatomical characters, the RI and CI of the two datasets were compared.

2.5 Functional and ecological correlates

In order to simplify the root anatomical variation and to search for one or several "functional root anatomical ~~traits~~syndromes", a non-metric multidimensional scaling (NMDS) was calculated using the metaMDS function. Because both categorial and continuous variables were used, the axes were extracted from the Gower distance matrix, using Jaccard distances for the categorial variables (which were first transformed to presence / absence values) and Manhattan distance for the standardized continuous variables. Presence / absence variables have the advantage that variation in the data can be retained. Variation in the functional root anatomical ~~traits~~syndromes was compared to the edaphic habitats (well-drained, groundwater, or wet/impeded drainage, scored subjectively from extensive fieldwork and herbarium labels), average mean annual precipitation, and average seasonality per species (extracted from the CHELSA database (Karger et al., 2017)~~)~~. These variables were selected as they can be assumed to impact on root function. The contribution of each anatomical character to each functional ~~trait~~syndrome was estimated using Spearman correlations (these were mostly scored as binary characters), and the ecological correlates of each explored with multiple linear regressions. In order to explore the evolution of the functional ~~traits~~syndromes, the optimal model (Brownian Motion or the Ornstein-Uhlenbeck process), was determined. Subsequently phylogenetic regressions were calculated to determine whether environment rather than phylogeny explained the variation in the four functional ~~traits~~syndromes, using as model for the co-variance an Ornstein-Uhlenbeck model, with a fixed root (it seems to make no difference using a random root). The analyses were made in R (R Development Core Team, 2017~~)~~, using the packages ~~"~~vegan~~"~~ (Oksanen et al., 2012), "Phytools" (Revell, 2012), "ape" (Paradis et al., 2004) and "phylolm" (Ho and Ane, 2014).

3 Results and ~~D~~discussion

The root anatomical descriptions, illustrations of the root anatomy of most species, detailed descriptions of the characters used, and the dataset in searchable format, are available in the Delta Intkey format at (http://www.systbot.uzh.ch/en/Bestimmungsschluessel/Restionaceae.html).

3.1 Anatomy and ontogeny

3.1.1 Plan

All roots investigated followed the same basic plan. I recognize, from outside to inside: a unicellular rhizoderm (= epidermis, Raechal and Curtis, 1990), unicellular exoderm, a multicellular cortex divided into three zones, a unicellular endoderm,

1–10 cellular pericycle, vascular bundles (= fascicles), separated into separate phloem and xylem (metaxalem), embedded in a ground tissue, and with the bundles often enclosed in distinct cells.

The apical 2–6 cm of the roots are white and soft, with little evidence of lignification. This probably matches the “fine root” region, although the roots in the restios in this region can have a wider diameter than the older roots, where there has often been a collapse of the cortex. It is also referred to as the root hair zone, and although all restio species studied show root hairs in this region, these often persist into the mature portion of the root. The “mature” part of the root is brown, hard, mostly well lignified. In most roots studied the cortex is not intact in this part. All cell layers are formed at the very tip of the root, and within the first centimeter of the root the stele (with the primary vascular tissue and the metaxylem), the cortex, and the rhizoderm is differentiated (Fig. 1A, E). The root diameter, measured from the endodermis, varies from 0.13—0.66—2.34 mm.

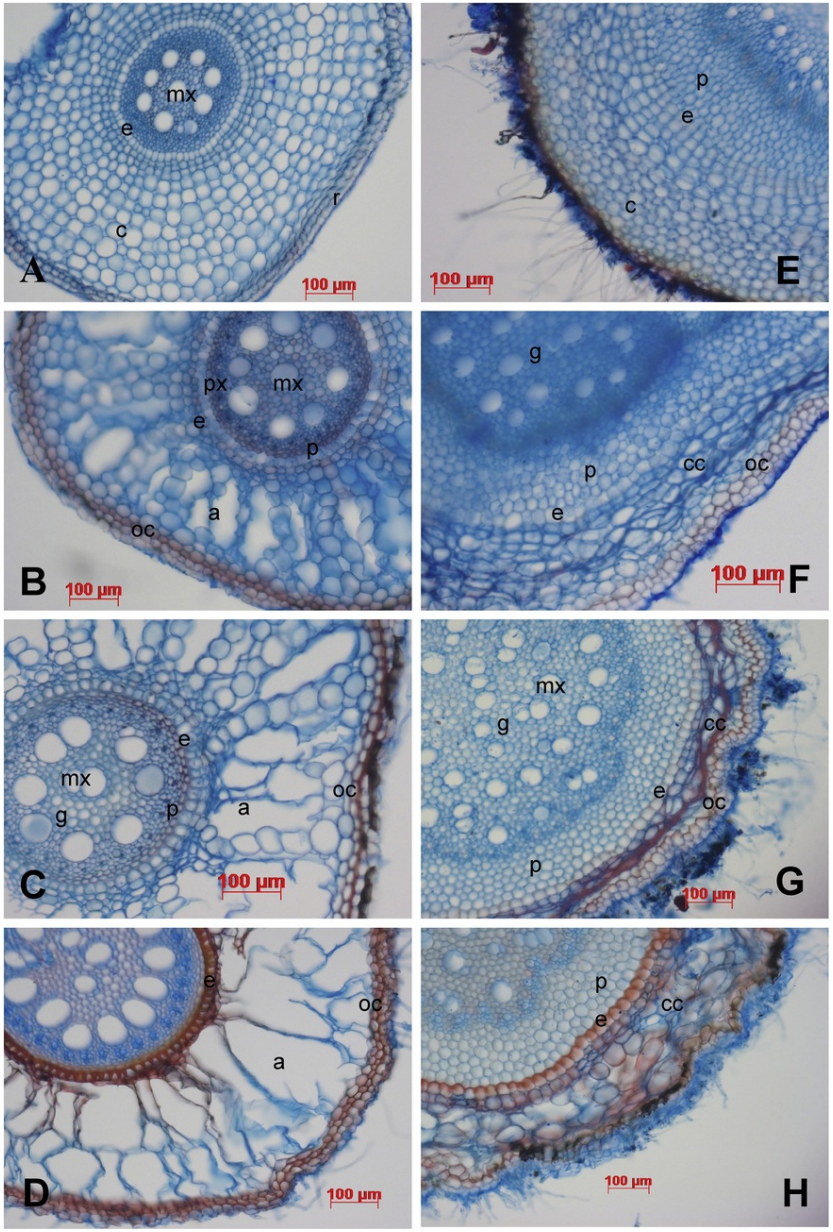
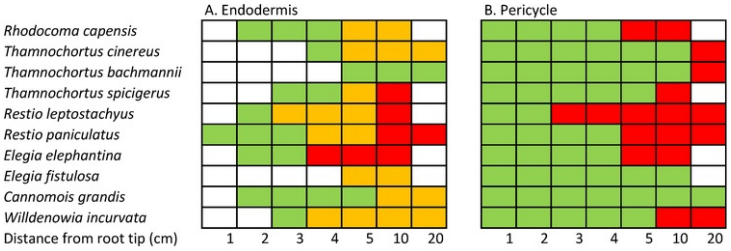


Fig. 1 Root ontogeny in *Elegia elephantina* (A–D) and *Thamnochortus cinereus* (E–H). A, E: 1 cm from root-tip; B, F: 3 cm from root-tip; C: 4 cm from root-tip; D: mature, G: 8 cm from root-tip; H: 12 cm from root-tip. Annotations: a = aerenchyma, c = unmodified cortex, cc = crushed cortex, e = endodermis, oc = outer cortex, p = pericycle, s = stele with metaxylem, mx = metaxylem vessels.

alt-text: Fig. 1

3.1.2 Rhizoderm

The single cell-layered rhizoderm bears the roothairs. It is always thin-walled and often poorly preserved on herbarium specimens, but there is no evidence of it being sloughed off. The size of these cells, relative to the exoderm, is variable, and in a few species they are much larger than the exoderm or cortical cells. However, not enough high-quality observations are available to interpret these observations. The rhizoderm appears always to be suberized, lighting up under fluorescence, and that from the very tip of the root (Fig. 2A). Roothairs are often persistent well beyond the root-hair zone, but the association between roothair persistence and sand-sheaths, as documented for the Australian Restionaceae (Pate and Delfs, 1999), was not explored here.



a. Endodermis: green = differentiated from pericycle, pale brown = lignified, red = sclerified and visibly thickened
b. Pericycle: green = visible, red = sclerified/lignified

Fig. 2 Comparative ontogeny of restio root epidermis. White means no observations.

alt-text: Fig. 2

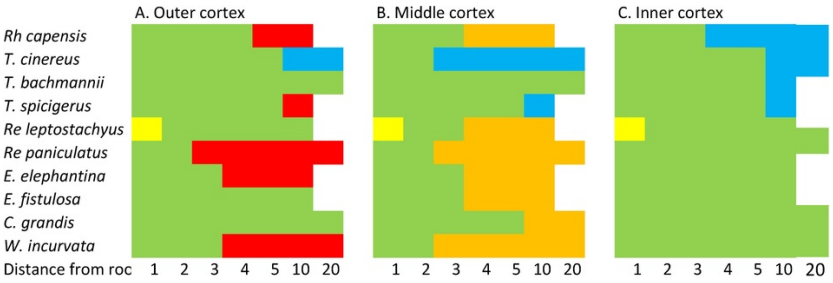
Raechal and Curtis (1990) reported much variation in this layer in wall thickness, cell size, and cell shape. A velamen, which is widespread in Asparagales (Kauff et al., 2000), was not described for Poaceae (Goller, 1977) and is absent in Restionaceae (here, and Pate and Delfs, 1999).

3.1.3 Exoderm

The single-layered exoderm, directly below the rhizoderm and as such the second layer of cells, is highly variable. It, too, is rarely well preserved. The outer walls, abutting the rhizoderm, are always thin. The inner walls, abutting the cortex, are often thickened and stain red with safranin. Often there is a narrow brightly luminescent layer, possible suberin. The wall itself does not fluorescence nor diffract polarized light. This is initiated early in the root development, generally 1 cm from the tip (Fig. 2B). Such a suberin layer has also been documented for the grasses (Goller, 1977) and Asparagales (Kauff et al., 2000); in the latter group it can also be lignified. This, together with the outer zone of the cortex, might be associated with limiting radial oxygen loss, as shown by Armstrong et al. (2000) in *Phragmites*.

3.1.4 Cortex

During the fine root stage the cortex is parenchymatous, as in most other monocots (eg Asparagales, Kauff et al., 2000) (Figs. 1A, B, 3). Already in this immature stage the cortex can be differentiated into inner, middle and outer zones. These differ in cell packing and cell size. Such a differentiation is common in monocots, and was described in the Australian Restionaceae (Pate and Delfs, 1999). The only well substantiated monocot exception is for Hyacinthaceae (Sobotik and Speta, 1997). The middle cortex can be distinguished from the inner and outer cortex by its larger, less tightly packed cells (eg in *Restio leptostachyus, Willdenowia incurvata, Elegia fistulosa*). In most restios the cortex cells either collapse, forming a dense tissue of crushed cell walls (e.g. *Thamnochortus*, Fig. 1F), or form an aerenchyma. Lynch et al. (2014) suggest that there is a substantial cost to retaining an unmodified root cortex.



Green = cells distinct; red = sclerified; brown = lysigenous; blue = collapsed

Fig. 3 Comparative ontogeny of restio root cortex. Rh. = *Rhodocoma*, T. = *Thamnochortus*, Re = *Restio*, E. = *Elegia*, C. = *Cannomois*, W. = *Willdenowia*.

alt-text: Fig. 3

The outer cortex, in aerenchymatous species, often developes into 2–10 cell lines of usually somewhat sclerified cells, generally only after 3 cm (Figs. 1B–D, 3A). In a few species, this layer becomes thickened, forming stone cells, sometimes with cells with massive C-shaped thickening in the outer layers of the outer cortex. The number of cells involved, and the fate of the cells, appears to be a generic trait. In *Elegia*, for example, there are almost always c. 3 lines of markedly thickened cells, separating the aerenchymatous middle cortex from the exodermis (i.e. Fig. 1D). Such a sclerified outer cortex appears to be associated with an aerenchyma, and forms a ring between aerenchyma and exodermis. In these instances the outer cortex is sharply distinct from the exoderm. In a few cases it almost looks as if the exodermis might be several cell-lines deep. This outer layer may be what Kauff et al. (2000) referred to as a "periderm", and may be important as a barrier to oxygen loss (radial oxygen loss) (McDonald et al., 2002).

The aerenchyma develops from the middle part of the cortex (Fig. 1B) usually at about 3 cm from the roottip (Fig. 3B). The aerenchyma is always formed by lysigeny (by the dissolution of cells). At maximum development the cortex consists of wide open passages separated by columns or plates of cells, often only one cell wide, and sometimes reduced to a single wall. In the few species where ~~we~~I was~~ere~~ able to study the development of the aerenchyma (*Elegia fistulosa, Restio leptostachyus*), the cavities formed by the collapse of cortical cells, leaving a few to make ~~a make~~ the scaffolding still seen in older roots. Such aerenchyma formation has been reported from many groups, such as the Bambusoideae (Raechal and Curtis, 1990). However, in most restio species the aerenchyma is formed by small passages separated by intact regions many cells wide. In older roots the whole cortex tends to collapse, leading to a chaotic mix of cavities and cells. This was observed in *Willdenowia incurvata*, where the young roots had clearly formed cavities, whereas the older roots show this mix of cavities and cells. This may be difficult to separate from a collapsed cortex. The diameter of the aerenchyma (thus the proportion of the root diameter it constitutes) is very variable. The area of the aerenchyma relative to the diameter of the stele might be related to the efficacy of oxygen transport to root tips (McDonald et al., 2002).

The inner part of the cortex, abutting the endodermis, is usually (but perhaps not always) thinwalled. It generally starts with, in transverse section, oblong cells, smaller than either middle or outer cortex, and quite tighly packed. In mature roots these cells may remain as a separate, parenchymatous layer, or collapse, or become lysignous and form part of the aerenchyma. Cell collapse can be seen to happen at 3 cm from the roottip in *Rhodocoma capensis*, but later in *Thamnochortus* (Fig. 3C). The cells collapse, the walls are crushed together, and in the end only a compacted tissue of cell walls can be seen. At least in *R. capensis* these cells were thin-walled before they were crushed. Starch deposition, as observed in the Australian restios (Pate and Delfs, 1999) was not observed in any African restios.

The structure of the cortex may be an interesting character separating families. In *Orectanthe* Maguire (Xyridaceae) the cortex of the young roots, when they are still absorptive, is undifferentiated, as in restios. With age the cortex differentiates into three zones, the inner of which forms an aerenchyma by the collapse of the radial walls (de Oliveira et al., 2015). The structure in *Abolboda* Bonpl. is similar (Scatena et al., 2011).

3.1.5 Endodermis

The endodermis is always visible in mature roots as a single ring of cells, differentiated from both the cortex to the outside and the pericycle to the inside. It is a useful marker relative to which the positional homology of tissues can be assigned. In young roots there is no difference between pericycle and endodermis cells, this difference develops later, mostly about 1–3 cm behind the tip of the root (Fig. 4A). Theoretically, the endodermis should always be visible by the Casparian strip, but our staining methods did not reveal it. It could sometimes be seen with DIC optics as a diffraction zone along the tangential walls. An apparently double endodermis is found only in *Hydrophilos rattrayi* (Pillans) H.P.Linder, here the outer "endodermis" is much smaller than the inner, and square rather than elongated.

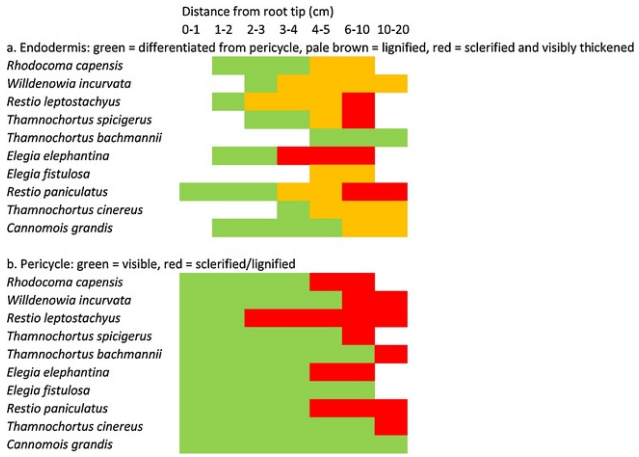


Fig. 4 Comparative ontogeny of restio root endodermis and pericycle.

alt-text: Fig. 4

The endodermis cells are enormously variable in size, shape and thickening. In size, relative to the pericycle, they range from less than ½ the volume of the pericycle cells to more than x2 larger. The size differentiation between endodermis and pericycle develops more or less at the same time as the aerenchyma. The cell shape ranges from wider than tall (extended periclinally) to more than twice as tall as wide (extended radially). The cell wall thickening is equally bewilderingly complex. This develops late in the root ontogeny. Near the tip of the roots (2–3 cm from the apex) the endodermis starts differentiating from the pericycle. The thickening only starts when the endodermis cells have reached their full size, and the thickening starts with lignification, at about 4 cm from the tip (Fig. 4A). Sclerification and the formation of thickened cell walls is seen only in brown roots, and this thickening may continue as the roots age. Consequently, it is still unclear how much of the interspecific differences may be due to the age of the root. A gradual thickening of the endodermis with increasing root age was also reported for the asparagoid *Behnia* (Conran, 1999) and in the Poaceae (Goller, 1977). Endodermis is sometimes grouped in three categories: primary (the walls unthickened, only a Casparian strip present), secondary (walls suberized but not thickened) and tertiary (walls thickened) (Goller, 1977; Kauff et al., 2000). This more or less matches the types of thickening I used here:

- The simplest form has unthickened walls. In this state only the Casparian strip is present. This matches the primary state. This is rare in restios, and by far the most common state in Hyacinthaceae (Sobotik and Speta, 1997).
- Next in complexity is that only the inner wall is thickened. This can range from almost the same as the outer wall, to filling more than 2/3 of the cell lumen.
- The lateral walls may be unthickened, and if thickened then almost always more in the inner than the outer side. Very heavily thickened lateral walls can occlude the inner part of the cell lumen, so forming a V-shaped structure; less massively thickened lateral walls, in conjunction with thickened inner walls, form a U-shaped structure.
- The outer wall is almost never thickened. However, in a very few species all four walls are equally and slightly thickened.

A similar massive variation in the thickening was also reported for Bambusoideae (Raechal and Curtis, 1990) and Asparagales (Kauff et al., 2000).

3.1.6 Pericycle

The pericycle consists of, in transverse section, more or less round cells, and is 1—15 cells deep and 10—71—272 µm wide. The number of cells in the pericycle seems to be conserved, these are formed at the root tip. In most plant groups the pericycle is simple, in Asparagales a multilayered pericycle is rare (Kauff et al., 2000). However, it can be many-celled in Poaceae (Goller, 1977), suggesting that the tendency to a multilayered pericycle may be a Poalean characteristic.

Pericycle cells are initially parenchymatous, and sclerification happens relatively late, generally more than 4 cm from the root tip (Fig. 4B). The wall thickening in the pericycle ranges from absent to massive with small channels visible in the walls, and almost occluding the lumen. Generally, the pericycle cells are all the same, from the endodermis to the ground tissue, but in a few cases the thickening or size may change radially. The pericycle is the cambial tissue from which the lateral roots originate. According to Goller (1977) the loss of this cambial capacity is indicated by the sclerification of the pericycle cells. A massive sclerification of the pericycle is also found in the Poaceae (Goller, 1977). Very similar variation in pericycle thickening was also reported for Bambusoideae (Raechal and Curtis, 1990), with the difference that in bambusoids there can also be thickening opposite phloem poles, a pattern we did not see in restios.

3.1.7 Ground tissue and stele

The vascular bundles are embedded in the ground tissue. These cells are initially parenchymatous, and show evidence of lignification usually synchronous with the pericycle, 4–6 cm behind the root tip. This is structurally quite similar to the pericycle, but is almost never identical to it. It may differ in cell size, in the degree of cell wall thickening, and often in the stain colour of the cells walls. In general, the ground tissue cells are largely homogenous from outside to inside, but there are instances of islands of thickened cells, or a gradient in wall thickening. Most species have somewhat thickened ground tissue cells, this is common in monocots, but absent from Hyacinthaceae (Sobotik and Speta, 1997). The sclerification of the ground tissue tends to be synchronous with that of the pericycle.

3.1.8 Vascular bundles

The primary vascular tissue is generally a ring directly inside the pericycle, and consists usually of 10–30 bundles of alternating phloem and protoxylem. These are already distinct within 1 cm from the root tip, and are very soon lignified, generally also within the first centimeter from the root tip (Fig. 5B). Generally, the protoxylem is constructed of few elements, often only one in transverse section. As the ground tissue sclerifies with increasing root age, the primary vascular bundles become isolated in the ground tissue.

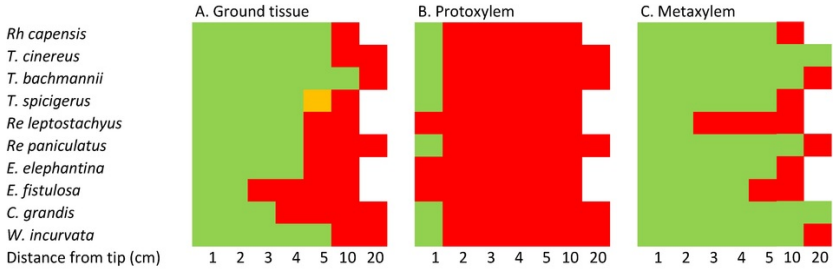


Fig. 5 Comparative ontogeny of the restio stele and vasculature: A: ground tissue, B: protoxylem, C: metaxylem. Rh. = *Rhodocoma*, T. = *Thamnochortus*, Re = *Restio*, E. = *Elegia*, C. = *Cannomois*, W. = *Willdenowia*.

alt-text: Fig. 5

The metaxylem is formed on the inside of the protoxylem within the first centimeter from the root tip. However, lignification is surprisingly late, mostly more than 5 cm from the root tip (Fig. 5C). The metaxylem poles are mostly single, rarely several clustered, metaxylem cell(s). Visually dominant are the large metaxylem vessels, whereas the protoxylem poles are more difficult to observe. The pole sheath, a single ring of cells, may be identical to the ground tissue, or may be thinner-walled (sometimes parenchymatous), and often elongated. The metaxylem vessels vary in three ways. The arrangement of the vessels can be simplistically categorized as a ring or scattered. However, there are cases where the “scattered” vessels give the impression of being organized into two rings. It is not clear whether this is accidental, or real organization into two rings. A second variation is when almost all vessels are in a ring, but some lie outside the ring. Furthermore, where there are fewer than five vessels, it is not possible to determine whether they are in a ring or scattered. The number of vessels ranges from 2-200. These correlate with the arrangement, with a ring arrangement with fewer vessels, and scattered more numerous. All or almost all species are polyarch. Although Goller (1977) suggested that the number of metaxylem vessels correlates with the diameter of the roots, in restios the organization of the metaxylem may be a more important variable. Both number and arrangement of vessels is fixed within the first centimeter of the root. Finally, the size of the vessels ranges from almost indistinguishable from the ground tissue cells (eg *Elegia squamosa* Mast., a trait probably restricted to a few species in *Elegia*), to c. 5 cells more or less filling the stele (eg *Platycaulos acutus* Esterh.), the vessel diameter ranges from 11—47—147 µm. This variation in metaxylem vessel characters is similar to that reported for the bambusoids by Raechal and Curtis (1990).

3.2 Phylogeny

3.2.1 Phylogenetic constraints

Mapping of anatomical traits shows that most are conservative on the phylogeny (the mappings are shown in Supplementary information), and testing them against a null distribution shows that 20 characters show strong, two weak, and three no phylogenetic pattern (Table 1). This is consistent with most previous reports, which suggest taxonomic pattern (or phylogenetic constraint) in the variation of old root anatomy. The consistency index, retention index and rescaled consistency index covary with the significance testing results. Combined, these show that the most phylogenetically informative characters are the organization of metaxylem (char. 1) (Fig. 6), the number of pericycle layers (chars 4, 5), and whether the cortex is differentiated (char. 14) leading to an aerenchyma (char. 17).

Table 1 Summary of restio root anatomy traits and statistics when mapped over the MCC phylogenetic hypothesis. Model = character model: Ord = ordered (additive), Unord = unordered; C.I. = consistency index; R.I. = retention index; R.C. = rescaled consistency index. The five characters that fit the phylogeny closest are shaded.

alt-text: Table 1

Char	Description	Model	Steps	Null	P	C.I.	R.I.	R.C.
1	Metaxylem organisation	Ord.	152	290-370	***	0.487	0.791	0.385
2	Number of metaxylem cells	Ord.	345	391-448	***	0.267	0.339	0.09
3	Protoxylem organisation	Ord.	38	45-52	***	0.079	0.286	0.022
4	Pericycle (< 3 > cells)	Ord.	56	83-102	***	0.429	0.6	0.257
5	Number of pericycle cells	Ord.	490	549-588	***	0.692	0.441	0.305
6	Pericycle cell wall thickness	Ord.	169	181-211	***	0.42	0.324	0.136
7	Ground tissue compared to pericycle	Ord.	102	102-128	***	0.304	0.398	0.121
8	Ground tissue cell walls compared to pericycle	Ord.	69	78-103	***	0.319	0.44	0.14
9	Ground tissue cell size compared to pericycle	Ord.	59	78-104	***	0.186	0.525	0.098
10	Ground tissue islands of lignified cells present	Unord.	5	5	NS	0.4	0.0	0.0
11	Endodermis cell size compared to pericycle	Ord.	145	169-193	***	0.421	0.408	0.172
12	Endodermis cell shape in TS	Ord.	123	132-152	***	0.358	0.275	0.098
13	Endodermis cell wall thickness	Ord.	213	225-249	***	0.488	0.248	0.121
14	Cortex undifferentiated or 3-layered	Unord.	53	88-114	***	0.491	0.716	0.352
15	Undifferentiated cortical cell wall thickness	Unord.	57	51-67	NS	0.211	0.211	0.045
16	Cortex inner layer wall thickness	Unord.	51	46-53	NS	0.412	0.063	0.026
17	Cortex middle layer structure (aerenchyma)	Unord.	51	59-72	***	0.471	0.438	0.206
18	Cortex middle layer width compared to stele	Ord.	146	163-194	***	0.363	0.415	0.151
19	Cortex outer layer cell wall thickness	Ord.	74	68-88	**	0.351	0.238	0.084
20	Cortex outer layer of number of cells	Ord.	34	34-39	***	0.529	0.238	0.126
21	Exodermal cell wall thickness compared to cortex	Ord.	140	141-180	***	0.079	0.339	0.027
22	Exodermal cell size compared to cortex	Ord.	75	84-99	***	0.173	0.287	0.05
23	Rhizodermal cell wall thickness compared to cortex	Ord.	32	29-36	*	0.063	0.118	0.007
24	Rhizodermal cell size compared to cortex	Ord.	80	108-134	***	0.063	0.464	0.03

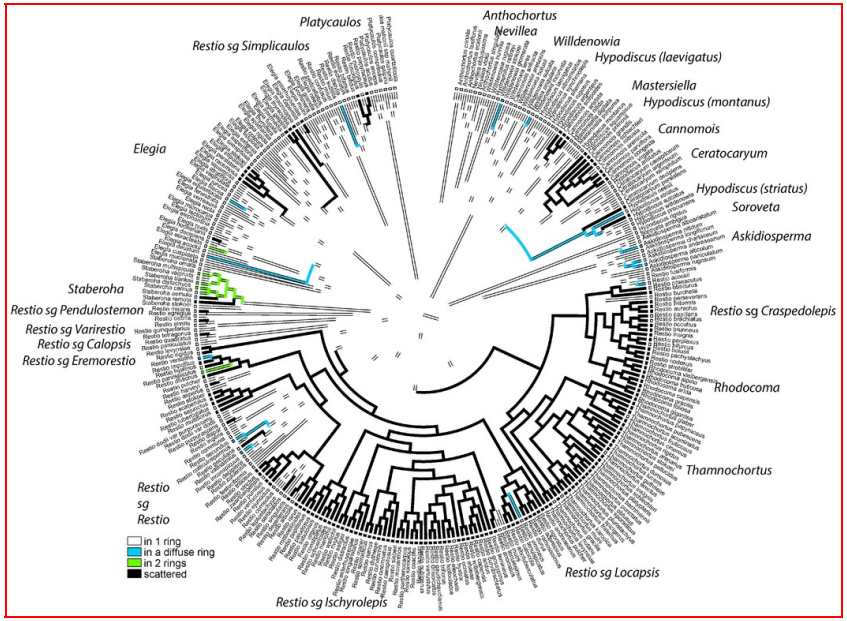


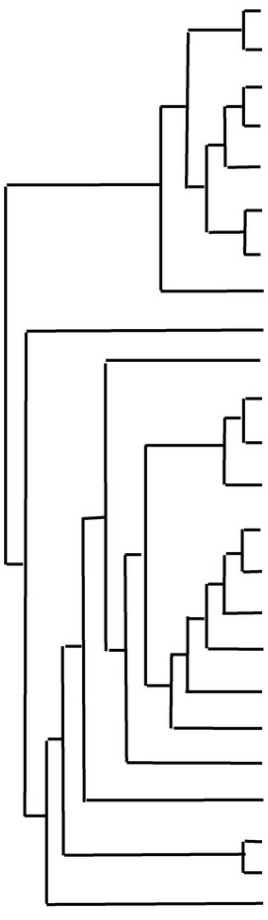
Fig. 6 Organisation of metaxylem mapped over the MCC phylogeny of the restios, using parsimony. The ancestral condition is inferred to be the metaxylem in a single ring. Scattered metaxylem evolved at least 14 times, and was lost at least 6 times. However, it characterizes two large clades (*Restio* L. subgen. *Craspedolepis* (Steud.) H.P.Linder & C.R.Hardy, *Rhodocoma* Nees, *Thamnochortus* P.J.Bergius), and also much of the rest of *Restio* (subgenera *Locapsis* H.P.Linder & C.R.Hardy, *Ischyrolepis* (Steud.) H.P.Linder & C.R.Hardy, *Restio, Eremorestio* H.P.Linder & C.R.Hardy). Subgenera and taxa referred to in the text are marked.

alt-text: Fig. 6

No root anatomical characters fit perfectly on the phylogeny (i.e. with CI or RI = 1), so we find no irreversed synapomorphies. Furthermore, most characters are variable within each group, as is illustrated for the metaxylem organization in Fig. 6. Although most root anatomical characters are phylogenetically constrained, some are more constrained than others. This is similar to the patterns obtained for culm anatomical variation. The comparison of the culm and root anatomy CI and RI values revealed similar distributions, with no significant differences according to the Wilcoxon Rank Sum test. Comparisons of the distributions of the values (see histograms in the Supplementary Information) show that in the culm anatomy there are more characters with a very poor fit to the phylogeny, and some that fit the phylogeny perfectly, so culm anatomy has a tendency to produce more phylogenetically useful characters.

3.2.2 Phylogenetic patterns

Optimisation (Fig. 7) indicates that the ancestral African restio had the metaxylem organized in a ring, a pericycle of three cells (compared to the common monocot pattern of 1 pericycle ring), the endodermal cells about twice a large as the pericycle, square and very heavily thickened, a cortex with aerenchyma and outer cortical ring thickened and about the same size as the exoderm. Several broad patterns can be distinguished. *Restio*, *Rhodocoma* and *Thamnochortus* are characterized by scattered metaxylem vessels; nested in this group the *Restio* subgenera *Locapsis*, *Ischyrolepis* and *Restio* generally have heavily thickened pericycle. This is constrasted to the thin-walled (and few-celled) pericycles typically found in *Restio* subgen. *Simplicaulos* H.P.Linder & C.R.Hardy and its phylogenetic sister, *Elegia* L. Within the Willdenowieae, *Willdenowia* Steud., *Hypodiscus* Nees, *Cannomois* Beauv. ex Desv. and *Ceratocaryum* Nees share a pericycle with more than three cells, and an endodermis the same size as the pericycle.



Taxon	Metaxylem	Pericycle cell number	Pericycle thickening	Endoderms to pericycle	Endodermis shape	Endodermis thick	Cortex	Outer cortex
Ancestral	ring	<4	med	X2	square	V	aer	thick
Anthochortus		4+						
Nevillea			thin					
Willdenowia		4+		equal		U		
Hypodiscus (la)		4+		equal		U		
Mastersiella		4+		equal		U		thin
Cannomois	scat	4+		equal	wide	U		thin
Ceratocaryum		4+	thin	equal		U		thin
Hypodiscus (st)		4+		equal				thin
Soroveta			thick		tall			
Askidiosperma			thin	> x2		U		
Craspedolepis	scat	4+		equal		U	col	thin
Rhodocoma	scat	4+		equal		M	col	thin
Thamnochortus	scat	4+		equal		U	col	
Locapsis	scat	4+	thick	equal	wide	U	col	thin
Ischyrolepis	scat	4+	thick	equal	wide	U		thin
Restio	scat	4+	thick	equal		U	col	thin
Eremorestio	scat	4+		equal	wide	U		thin
Calopsis		4+				U		
Varirestio	scat	4+	thick		wide	U		thin
Pendulostemon		4+				U		thin
Staberoha	scat	4+	thick	equal		M		thin
Elegia			thin		tall	U		
Simplicaulos		4+	thick			M		
Platycaulos				> x2				

Fig. 7 Summary of root anatomy evolution by clade (genus or subgenus of *Restio*). The phylogenetic tree has been simplified to the clade tips. Mx = metaxylem, PC = pericycle, ED = endodermis, CGT = central ground tissue, endodermis thickening (V = massive, U = normal, M = minimal), Cortex (Aer = aerenchyma, Col = collapsed), outer cortex (thick = thickwalled, thin = thinwalled), outer cortex size (small: < than rhizoderm; large > or = than rhizoderm). **Bold, red** = all species in clade have this attribute; underlined, black = ancestral state, but reversed so not all species have this trait; green = not ancestral state, but most species in clade with this state; blue = ancestral but fewer than half of the species with this trait (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

alt-text: Fig. 7

3.2.3 Functional ~~traits~~syndromes

In order to achieve convergence for the non-metric multidimensional scaling, four dimensions had to be extracted, with a very good stress value of 0.1185. These four dimensions are treated here as functional root anatomical ~~traits~~syndromes. The

first dimension (functional ~~trait~~syndrome) is positively correlated with the size of the roots: stele diameter, sum of the diameters of the vessels, the area inside the endodermis, and weaker with the number of vessels and the size of the individual vessels. This largely reflects the Willdenowieae, often found in habitats with ground water. Possibly large roots and large vessels are more efficacious in bringing water up from deep underground. The second functional ~~trait~~syndrome is correlated with the presence of aerenchyma, a thick outer cortex, metaxylem arranged in a ring, and a wide pericycle. This ~~trait~~syndrome is most common in genera found in wetlands, mostly in the genera *Elegia, Platycaulos, Soroveta* and *Hydrophilos*. These root traits may be interpreted to be associated with dealing with anoxic conditions, with aerenchyma contained by a thick outer cortex, possibly to reduce radial oxygen loss. The third functional ~~trait~~syndrome is corrolated with square endodermis cells and a collapsed cortex, but there is no obvious phylogenetic pattern. The fouth functional ~~trait~~syndrome also lacks a phylogenetic ~~/~~ systematic pattern.

A linear regression showed the first two functional ~~traits~~syndromes are significantly explained by soil moisture and mean annual precipitation (Table 2), the third functional ~~trait~~syndrome weakly by mean annual precipitation, and the fourth functional ~~trait~~syndrome by no environmental parameters. Rainfall seasonality did not significantly explain any root anatomical variation.

Table 2 Variation in the root functional ~~traits~~syndromes explained by selected environmental parameters. Lm = linear model without taking the phylogeny into account, plm = phylogenetically corrected linear (Caption to Table 2: change "MAP=Mean annual precipitation" and change "root functional trait" to "root functional syndrome") model. MAP = Mean annual precipitation; Seasonality = rainfall seasonality. R^2 of the regression model, p = whether the model explains a significant part of the variation.

alt-text: Table 2

	Functional trait 1		Functional trait 2		Functional trait 3		Functional trait 4	
	lm	plm	lm	plm	lm	plm	lm	plm
MAP	**	*	***	ns	*	**	ns	ns
Seasonality	ns	ns	ns	ns	ns	ns	ns	ns
Soil drainage	***	*	***	**	ns	ns	ns	ns
R^2	0.081	0.084	0.183	0.186	0.011	0.022	0.003	0.008
p	***	*	***	ns	ns	ns	ns	ns

The best model fit for all four functional ~~traits~~syndromes on the MCC phylogeny is the Ornstein-Uhlenbeck process (Supplementary ~~i~~information, Best Root Evolutionary Model). Due to the substantial differences in the AICc I did not recalculate them over a set of trees. The first two functional ~~traits~~syndromes covary strongly with the phylogeny, the third and fourth functional ~~traits~~syndromes hardly. The phylogenetic regression showed that the first two functional ~~traits~~syndromes were weakly explained by environmental variables, in the first functional dimension both MAP and soil drainage, and in the second only soil drainage, but that more strongly. The third functional ~~trait~~syndrome was explained by mean annual precipitation. The fourth showed no pattern at all.

This suggests that most of the variation in the root functional ~~traits~~syndromes (and implication the root anatomy) is explained by the phylogeny, and that the environment contributes little. However, the significant non-phylogenetic linear regressions indicate that although the root anatomy is largely phylogenetically constrained, the phylogeny is environmentally constrained, and that clades with particular root functional ~~traits~~syndromes are found in particular soil drainage and rainfall regimes. This “pattern” is not statistically significant due to the low number of transitions between soil regimes.

3.3 Species descriptions and generic diagnoses

Species descriptions can be generated with the Restionaceae Intkey tool at http://www.systbot.uzh.ch/en/Bestimmungsschluessel/Restionaceae.html. This tool can also be used for species identification. In trial runs some species can be identified, in most cases the interactive key brings the user to within a few, closely related, species.

In the generic diagnoses below the most useful characters are in bold,

3.3.1 Anthochortus Endl.

Cortex either without or with a fan-shaped aerenchyma. Endodermis cells square or tall, thickened to massively thickened, equally the adjacent pericycle cells (shorthand for cell-layers, this is used throughout the descriptions) are much bigger, stele (including endodermis) 300—376—440 µm in diam. Pericycle of 1~~—~~–4 unthickened, or somewhat thickened, cells, 20~~—~~–40 µm wide. Ground tissue cells usually larger and thinner-walled than pericycle cells; metaxylem vessels 8~~—18, always in a ring, 12~~ ~~—~~–18, always in a ring, 12–36 µm in diam. Fig. 8A.

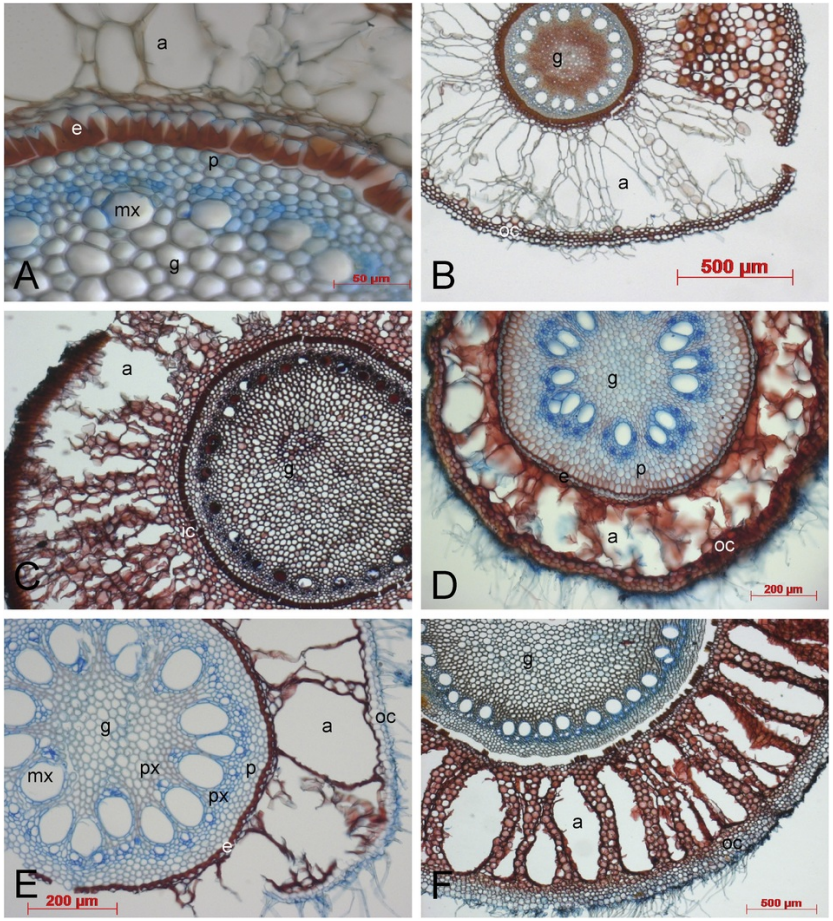


Fig. 8 A. *Anthochortus crinalis* (Mast.) H.P.Linder: thinwalled, 3-celled pericycle, and massively thickened, tall endodermis cells. B. *Hydrophilus rattrayii*: massive aerenchyma, thickened outer cortex. C. *Nevillea singularis* Esterh: thickened outer cortex and massively thickened endodermis. D. *Willdenowia sulcate* Mast.: pericycle enlarged and elongated towards outside, aerenchyma poorly developed. E. *Cannomois anfracta* H.P.linder: protoxylem scattered, metaxylem not strictly in one ring. F. *Ceratocaryum persistens* H.P.Linder: proto- and metaxylem strictly in one ring, pericycle thin-watted, outer cortex well developed. a = aerenchyma; c = cortex; cc = crushed cortex; ic = inner cortex; oc = outer cortex; e = endodermis; p = pericycle; s = stele; g = ground tissue; mx = metaxylem; px = protoxylem.

alt-text: F(Change: "D. *Willdenowia sulcata"* and a three lines lower, change "pericycle thin-watted" to "pericycle thin-walled")ig. 8

3.3.2 Hydrophilus H.P.Linder

Cortex with a fan-shaped aerenchyma, outer cortex of 1~~-3 thickwalled cells.~~ –3 thickwalled cells. Endodermis taller than wide, more than twice adjacent pericycle cells, massively thickened, stele c. 550 µm in diam. Pericycle of 2 cells, c. 24 µm wide, somewhat thickened. Groundtissue more or less similar to pericycle; metaxylem of 18 cells, diam. c. 48 µm, in a ring Fig. 8B.

Note. A single species, the root anatomy similar to that of *Anthochortus*, with few pericycle cells, massively thickened endodermis, and less than 20 metaxylem vessels.

3.3.3 Nevillea H.P.Linder

Cortex usually with a fan-shaped aerenchyma half as wide as stele, outer cortex thickwalled. Endodermis taller than wide, thickened to massively thickened, equaling to twice the adjacent pericycle cells, stele 185—1224 µm in diam. Pericycle 40 ~~–60 µm wide, cells 3—~~–60 µm wide, cells 3–4, thinwalled. Groundtissue usually larger than pericycle; metaxylem vessels 16—30, in a ring, diam. 60—80 µm Fig. 8C.

3.3.4 *Hypodiscus*

Cortex undifferentiated in *H. montanus* clade, and differentiated with a fanshaped aerenchyma about 1/8 stele width in *H. striatus* clade ("st" in Fig. 7), ½ stele width in *H. laevigatus* clade. Endodermis usually massively thickened (except *H. laevigatus*, *H. rugosus*, *H. argenteus*, *H. montanus*, *H. procurrens*, "la" in Fig. 7), usually square but also tall or wide, usually equally the adjacent pericycle cells, stele 470—1250 µm. Pericycle 18—275 µm wide, of 4–1250 µm. Pericycle 18–275 µm wide, of 4–8 unthickened, or somewhat thickened, cells. Ground tissue cells either similar to or larger than (in the *H. albo-aristatus* clade) the pericycle cells; metaxylem vessels vary between 20 and 100, usually in a ring, diam. 30—110 µm.

Note: this genus may be consist of two clades and a few outliers, the root anatomical data corroborate the plastid genome data.

3.3.5 *Mastersiella Gilg-Ben*

Cortex with a variably developed aerenchyma. Endodermis square to wide, thickened, equally the adjacent pericycle cells, stele diam. 620—1250 µm. Pericycle 30—70 µm wide, of 2—1250 µm. Pericycle 30–70 µm wide, of 2–6 unthickened, or somewhat thickened, cells. Ground tissue cells larger and sometimes thicker-walled than these; metaxylem vessels 16—35, diam. 45—35, diam. 45–85 µm, always in a ring.

3.3.6 *Willdenowia*

Cortex with a very variably-developed aerenchyma. Endodermis cells square to widened, very variously thickened, more or less equaling the adjacent pericycle cells, stele diam. 560—1670 µm. Pericycle 40—190 µm wide, of (2) 3—1670 µm. Pericycle 40–190 µm wide, of (2) 3–7 (10) usually heavily thickened, rarely unthickened, cells, often the outer rows massively thickened and elongated, and the inner rows isodiametrical and thin-walled, grading into the ground tissue cells. Ground tissue cells either similar, or smaller and thinner-walled than pericycle cells; metaxylem vessels 30—100 µm in diam., 12—100 µm in diam., 12–60, always in a ring Fig. 8D.

Note. The elongated pericycle cells are unique in the genus.

3.3.7 *Cannomois*

Cortex always with a fan-shaped aerenchyma, this to half as wide as the stele; outer cortex sometimes with several continuous rows, or an interrupted row, of heavily thickened cells, often with a C-shaped thickening. Endodermis cells usually wider than tall, thickened, and equally the adjacent pericycle cells, stele 640—1880 µm. Pericycle 40—220 µm, of 3—1880 µm. Pericycle 40–220 µm, of 3–11, unthickened, or somewhat thickened, cells. Ground tissue cells usually similar to these, if different then thicker-walled and larger; metaxylem vessels (16) 30—70 (200), almost always scattered (except *C. arenicola* and *C. anfracta*) Fig. 8E.

Notes: this is the only genus in the Willdenowieae with scattered metaxylem.

3.3.8 *Ceratocaryum*

Cortex always with aerenchyma, this sometimes fan-shaped, and sometimes of scattered cavities. Endodermis cells square or tall, thickened to massively thickened, more or less equaling the adjacent pericycle cells, stele diameter 1070—2350 µm. Pericycle 44—120 µm, of 3—2350 µm. Pericycle 44–120 µm, of 3–6 thin-walled cells. Ground tissue cells similar to these; metaxylem vessels 28—40, diam. 80—40, diam. 80–120 µm, usually scattered, always in a ring Fig. 8F.

3.4 *Soroveta* H.P.Linder & C.R.Hardy

Cortex with a fan-shaped aerenchyma as wide as the stele; endodermis tall, massively thickened, more or less equaling the pericycle cells, stele diam. c. 470 µm; pericycle c. 40 µm wide, of 2 heavily thickened cells; ground tissue cells similar to these; metaxylem vessels vary between 10 and 15, in a ring, diam. c. 45 µm.

3.5 Askidiosperma

Cortex always with a fan-shaped aerenchyma, this can be as wide as the stele; endodermis twice as big or more than adjacent pericycle cells, square to tall, thickened, stele diameter 336--1081 µm; pericycle 14--74 µm, of 3--5 unthickened, or somewhat thickened, cells; ground tissue cells either similar to, or larger than thicker-walled than the pericycle cells; metaxylem vessels vary between 10 and 50, usually arranged in a ring, 34—83 µm.

3.6 *Restio* subgen. *Craspedolepis*

Cortex almost always with a fanshaped aerenchyma (rarely absent, rarely made of cavities), up to as wide as the stele. Endodermis square or rarely widened, more or less thickened, equaling or twice as big as adjacent pericycle cells, stele 310—1050 µm in diam. Pericycle 18—180 µm wide, of 2–180 µm wide, of 2–7 usually somewhat, rarely heavily, thickened cells; ground tissue cells usually similar to the pericycle cells, if different then smaller and thinner-walled; metaxylem vessels 20—82 µm in diam., 8–82 µm in diam., 8–25 (80), almost always scattered, a ring organization is found in 4 species and evolved 3 times).

3.7 *Rhodocoma* H.P.Linder & C.R.Hardy

Cortex occasionally with aerenchyma, this can be either fanshaped or of cavities, and can be as wide as the stele; endodermis equaling the adjacent pericycle cells, square to occasionally wide, unthickened or thickened, stele 405–1200 µm in diam.; pericycle 50–250 µm wide, of 4–10 usually somewhat, rarely heavily, thickened cells; ground tissue cells usually smaller than, rarely similar to, the pericycle cells; metaxylem vessels 25–100, diam. 30–65 µm, always scattered Fig. 9A.

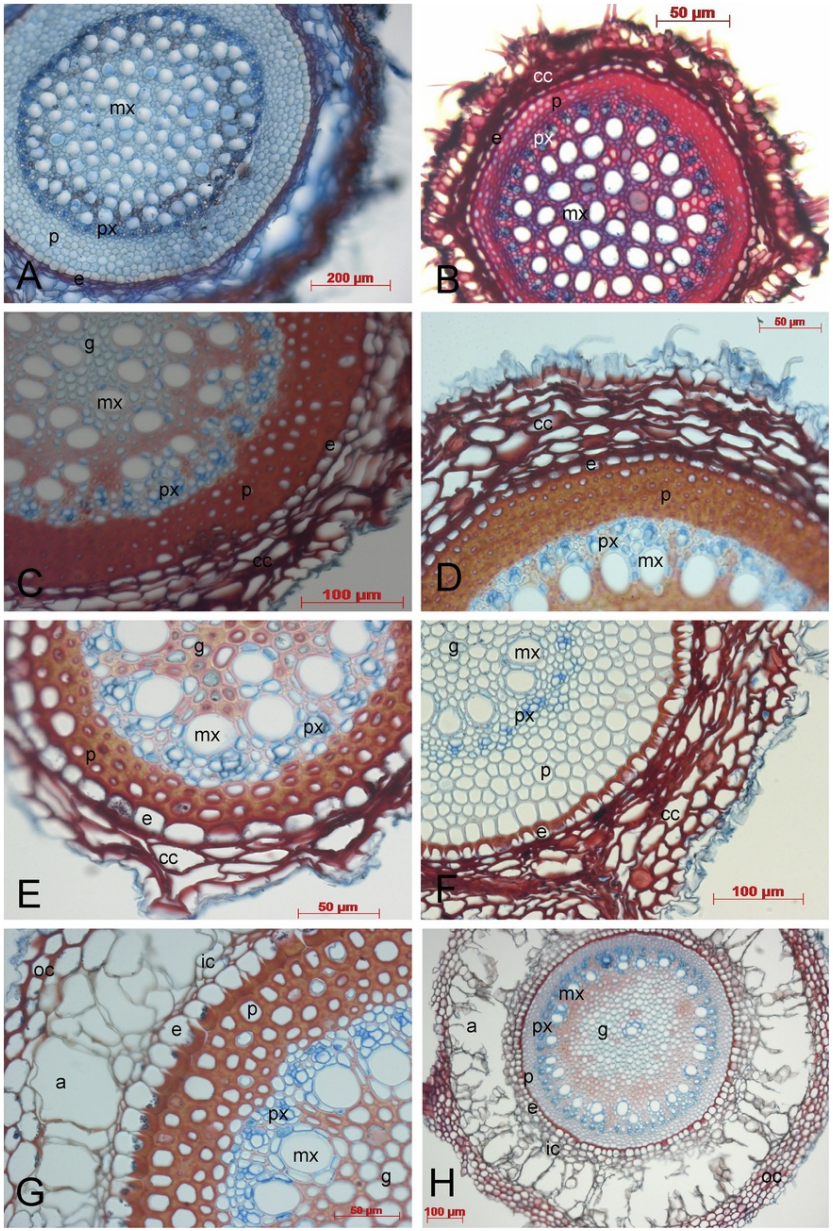


Fig. 9 Transverse sections of selected examples. A. *Rhodocoma arida* H.P.Linder & H.Vlok: note numerous metaxylem vessels, pericycle of 4–10 unthickened cells, and poorly developed aerenchyma. B. *Thamnochortus bachmannii*: cortex compressed, pericycle thickened. C. *Resti (Locapsis) rigoratus* (Mast.) H.P.Linder & C.R.Hardy: endodermis cells widened, cortex collapsed, pericycle 2–3 cells wide, thickened. D. *Restio (Ischyrolepis) cincinnatus* Mast. Pericycle 5 celled. E. *Restio (Restio) scaberulus* N.E.Br.: endodermis relatively thin-walled, large cells. F. *Restio (Restio) degenerans* Pillans: pericycle 5–6 almost thin-walled cells, endodermis square, with distinctly U-shaped thickening. G. *Restio (Restio) zuluensis* H.P.Linder: only species in the subgenus with aerenchyma and thickened outer cortex, note large thin-walled endodermis. H. *Restio (Calopsis) paniculatus*: aerenchyma well developed, outer cortex many-celled, pericycle few-celled. a = aerenchyma; c = cortex; cc = crushed cortex; ic = inner cortex; oc = outer cortex; e = endodermis; p = pericycle; s = stele; g = ground tissue; mx = metaxylem; px = protoxylem.

alt-text: Fig. 9

3.8 Thamnochortus

Cortex develops into a compressed, fibrous sheath; endodermis square to wide, thickened, and equalling the adjacent pericycle cells in volume, stele diam. 275–1200 µm. Pericycle 10—210 µm wide, of (2) 3–9 (11) usually somewhat, rarely heavily, thickened cells; ground tissue cells usually similar to pericycle cells, when different then smaller; metaxylem vessels 25–50 µm in diam., (10) 20–100, always scattered Fig. 9B.

Notes: *Thamnochortus* and *Rhodocoma* roots are quite similar, but differ by the occasional aerenchyma in *Rhodocoma.*

3.9 *Restio* subgen. *Locapsis*

Cortex usually undifferentiated and collapsing, but sometimes with a fan-shaped aerenchyma in *R. andreaeanus, R. durus* and *R. muirii*; outer cortex layer, when visible, thin-walled. Endodermis usually wider than tall, but square in two species, walls thickened (except in *R. durus* and *R. vimineus*), similar to or slightly larger than adjacent pericycle cells, stele 230–730 µm in diam. Pericycle 30–160 µm wide, of 3–160 µm wide, of 3–7 (10) usually heavily thickened cells (three species with only somewhat thickened cells). Ground tissue cells either similar or larger than pericycle cells; metaxylem vessels 20–40 µm in diam., 17–40 µm in diam., 17–70, always scattered (except possible *R. tenuispicatus*).

Notes: very similar to *Restio* subgen. *Ischyrolepis*, especially by the thickened pericycle, differs in the more consistently wide endodermis cells Fig. 9C.

3.10 *Restio* subgen. *Ischyrolepis*

Cortex either undifferentiated and collapsed or with a variously developed aerenchyma; outer cortex layer with up to 3 thin- or thickwalled cells. Endodermis cells square or widened, thickened, more or less equaling the adjacent pericycle, stele 196–1220 µm in diam. Pericycle 20–210 µm wide, of (2) 3–210 µm wide, of (2) 3–10 usually heavily, sometimes somewhat thickened, and only in *R. rivulus* thinwalled cells. Ground tissue cells either similar or dissimilar to pericycle cells; metaxylem vessels 19–60 µm in diam., (7) 14–100, scattered, in a ring only in *R. tenuissimus* and *R. anomalus* Fig. 9D.

Notes: the large subgenus with 55 species is remarkable by the diversity of form which the cortex can take.

3.11 *Restio* subgen. *Restio*

Cortex usually undifferentiated and collapsed, aerenchyma in *R. alticola, R. triticeus, R. zuluensis* and the *R. communis* – *R. purpurascens* clade, when present this is about 1/8 of stele width, and either fan-shaped or of cavities; outer cortex when differentiated thinwalled, thickwalled only in *R. rarus* and *R. purpurascens*. Endodermis square, rarely widened, tall only in R. zuluensis; usually thickened, rarely unthickened or massively thickened; more or less equaling the adjacent pericycle cells, stele 136–1680 µm in diam. Pericycle 20–170 µm wide, of (1) 2–170 µm wide, of (1) 2–7 (9) thin-walled to heavily thickened cells. Ground tissue cells either similar or dissimilar to pericycle cells; metaxylem vessels in diam. 18--85 µm, (2) 8–30 (50), scattered in four subclades and in a ring in three Fig. 7E–G.

Note: a tendency for metaxylem vessels to be in a ring, and fewer, than in subgen. *Ischyrolepis.* There are very distinct subgroups in root anatomy within this subgenus.

3.12 *Restio* subgen. *Eremorestio*

Cortex rarely differentiated with aerenchyma. Endodermis wider than tall, thickened, equaling the neighbouring pericycle cells, stele 190–800 µm. in diam. Pericycle 26–130 µm wide, of 2–130 µm wide, of 2–7 thickened cells. Ground tissue cells mostly similar to pericycle cells; metaxylem vessels 21–44 µm in diam., 8–44 µm in diam., 8–50, in diffuse rings or scattered.

3.13 *Restio* subgen. *Calopsis*

Cortex with a fan-shaped aerenchyma, up to as wide as stele; outer cortex of up to 3 thickened cells. Endodermis square, at least twice as large as adjacent pericycle cells, thickened, stele 390–680 µm. in diam. Pericycle 34

~~–45 µm wide, of 3-~~45 µm wide, of 3–4 thickened cells. Ground tissue cells either similar or thicker and larger than pericycle cells; metaxylem vessels 34~~–47 µm in diam., 15–~~47 µm in diam., 15–35, always in a ring Fig. 9H.

3.14 *Restio* subgen. *Varirestio* H.P.Linder & C.R.Hardy

The root anatomy of these three species is still inadequately known, and surprisingly variable. Cortex undifferentiated. Endodermis square to wide, variously thickened, smaller or larger than adjacent pericycle cells, stele 190––460 µm in diam. Pericycle 22~~–55 µm wide, of 4-~~55 µm wide, of 4–5 unthickened to heavily thickened cells. Ground tissue cells are either similar, or thinner-walled and larger than the pericycle cells; metaxylem vessels 27––43 µm in diam., vary from 10 to 30, usually arranged in a ring.

3.15 *Restio* subgen. *Pendulostemon* H.P.Linder & C.R.Hardy

Cortex always with an aerenchyma (not italic); outer cortex layer thick- or thinwalled, 1–3 cells thick. Endodermis from half to twice the size of pericycle cells, square to widened, thickened either only on inner wall, or also side walls, stele 250––1230 µm in diam. Pericycle 25~~–200 µm wide, of 2-~~200 µm wide, of 2–5 somewhat thickened cells. Ground tissue cells either similar or thinner and smaller than pericycle cells; the metaxylem vessels 25––100 µm in dia., vary between 10 and 15, arranged (usually) in a ring Fig. 10A.

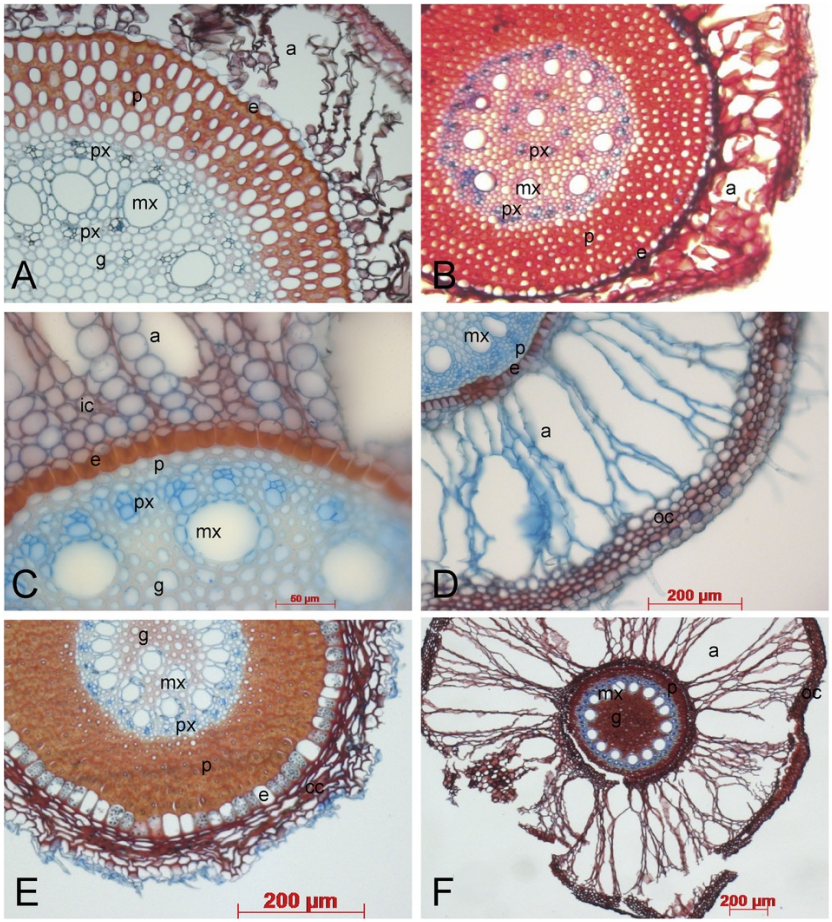


Fig. 10 Transverse old root anatomy of some Restionoideae clades. A. *Restio (Pendulostemon) egregious* Hochst. B. *Staberoha multispicula* Pillans: pericycle massive. C. *Elegia fenestrata* Pillans: pericycle 2 cells wide, endodermis massively thickened, tall. D. *Elegia prominens* Pillans: aerenchyma well developed, outer cortex of 3 thickened cell lines. E. *Restio (Simplicaulos) distylis* H.P.Linder & C.R.Hardy: pericycle massive, endodermis cells large. F. *Platycaulos quartizicola* (H.P.Linder) H.P.Linder & C.R.Hardy: massive aerenchyma. a = aerenchyma;

c = cortex; cc = crushed cortex; ic = inner cortex; oc = outer cortex; e = endodermis; p = pericycle; s = stele; g = ground tissue; mx = metaxylem; px = protoxylem.

alt-text: Fig. 10

(Caption to Fig 10: "*Restio (Pendulostemon) egregius*" and "*Platycaulos quartziticola*"]Notes: These two species differ remarkably in their root anatomy. They are isolated in *Restio*, and the root anatomy also suggests differentiation.

3.16 *Staberoha* Kunth

Cortex always with a fan-shaped aerenchyma about ¼ as wide as stele; outer cortex layer thinwalled, except in *S. stokoei* and *S. ornata*. Endodermis usually square, thickened only on the internal wall, equaling the pericycle cells (*S. stokoei* thick-walled, taller than wide, and bigger than adjacent pericycle cells), stele diam. 300–~~1000 µm. Pericycle 60—160 µm wide, of 5-~~1000 µm. Pericycle 60–160 µm wide, of 5–8, heavily thickened, cells, different from the thin-walled ground tissue cells (except *S. stokoei*, with only 1–2 unthickened cells). Ground tissue cells usually thinner walled than pericycle cells; the metaxylem vessels diam. 36–~~66 µm, 5—~~66 µm, 5–25, usually arranged in a double-ring, and in two species scattered, or sometimes appearing to be in one ring Fig. 10B.

The root anatomy of *Staberoha* is remarkably uniform, except with the first diverging, and also otherwise morphologically unusual Great Swartberg endemic *S. stokoei*. The genus is quite divergent in the family in Africa.

3.16.1 Elegia

Cortex, with the exception of *E. muirii*, differentiated into three layers, with the inner and middle layer forming an aerenchyma, from 1/8 of stele-width to wider than stele, the outer layer is thickwalled (except in *E. deustum* and *E. fistulosum*) and 2–3 cells thick. Endodermis either tall or square, thickened or massively thickened, usually at least twice the size of adjacent pericycle cells (except in *E. macrocarpa*, where it is the same size), stele 215–~~1180 µm. Pericycle 10—80 µm, of 1—~~1180 µm. Pericycle 10–80 µm, of 1–3 thinwalled or slightly thickened cells (except with *E. spathacea* with heavily sclerified pericycle cells). Ground tissue either similar to pericycle, or larger with thicker walls; the metaxylem vessels (7) 10–~~40 (45), diam. 26—~~40 (45), diam. 26–120 µm, usually arranged in a ring, but scattered in *E. racemosa* – *E. stokoei, E. galpinii-E. thyrsoidea, E. mucronata* and *E. esterhuyseniae*. Protoxylem is scattered in two small clades Fig. 10C, D.

The root anatomy of *Elegia* is quite divergent from the rest of the family.

3.16.2 Restio subgen. Simplicaulos

Cortex usually differentiated into three layers; inner and middle making an aerenchyma which varies from 1/8 to equalling stele width, outer cortex usually thickwalled; in two species collapsing in older roots. Endodermis square, thickened or unthickened, usually equalling the adjacent pericycle cells, stele 165–~~800 µm. Pericycle 20—140 µm, of (2) 3.~~800 µm. Pericycle 20–140 µm, of (2) 3–8 (10), often heavily thickened, cells. Ground tissue either similar to dissimilar to pericycle; the metaxylem vessels 25–~~70 µm in diam., 5—~~70 µm in diam., 5–25, usually in a ring, scattered in two species Fig. 10E.

Notes: the cortex is similar to *Elegia*, but *Restio* subgen. *Simplicaulos* differs from *Elegia* by more, and often heavily thickened, pericycle cells.

3.16.3 Platycaulos H.P.Linder

Cortex differentiated into three layers; inner and middle cortex forming a massive, fan-shaped aerenchyma, at least ½ as wide as the stele and often wider than the stele; outer cortex of 2–4 thickwalled cells. Endodermis cells square or taller than wide, usually more than twice as large as adjacent pericycle cells, and often massively thickened, stele 285–~~620 µm. Pericycle 15—45 µm wide, 1—~~620 µm. Pericycle 15–45 µm wide, 1–3 (4) somewhat thickened cells. Ground tissue cells usually of larger and more thick-walled than pericycle; the metaxylem vessels 9–~~22, 30—~~22, 30–80 µm in diam., strictly arranged in a ring Fig. 10F.

Notes: The root anatomy of *Platycaulos* is similar to that of *Elegia*.

4 Conclusions

Here we show that there is extensive variation in the anatomical characters of the older parts of restio roots. Much of this variation is phylogenetically structured (especially the organization of the metaxylem, the development of the pericycle, and the structure of the cortex). However, there is also extensive variation which appears not to be phylogenetically structured, at the current level of sampling it is not clear how much of this variation is the result of phenotypic plasticity, and how much of local adaptation. It is evident that root anatomy is not highly canalized. Despite (or maybe because) of this variation it is possible to identify roots to the correct species or species-group. However, this capacity still needs to be tested against a much larger data set.

It is evident that this root variation probably has ecological consequences, as was shown to be the case with the cortex development. The ecology – root anatomy correlation is, however, strongly phylogenetically constrained, complicating tests of adaptation. Furthermore, root anatomical variation could also be interacting with root architecture, and with the degree of development of fine root traits, such as cluster roots. These we will explore in upcoming

papers.

It is evident that the belowground organization of restios is variable, that this variation is to some extent phylogenetically structured, and to some extent is correlated to the habitats. It seems possible that the roots will hold the key as to which niches restios can occupy, how many species can co-exist, and which lineages have been successful.

~~Uncited referencesDevelopment and Team (2017), Seubert (1997), Seubert (1998a) and Seubert (1998b).Acknowledge~~Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2018.08.004.

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Appendix A. Supplementary data

The following are Supplementary data to this article:

Multimedia Component 1

Multimedia Component 2

Multimedia Component 3

Multimedia Component 4

Highlights

- African Restionaceae have extensive variation in their root anatomy.
- This variation is phylogenetically constrained.
- Groups of genera share a common anatomical plan.
- Species, or groups of species, can be identified by root structure.

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Query: Please Check Heading.
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Query: Have we correctly interpreted the following funding source(s) and country names you cited in your article: University of Zurich, Swiss Science Foundation; Claraz Foundation?

Answer: Yes